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# Intraspecific chemical variability of the leaf essential oil of *Juniperus phoenicea* subsp. *turbinata* from Corsica

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**Abstract**

The composition of 50 samples of essential oil of individual plants *of Juniperus phoenicea* subsp. *turbinata* from Corsica was investigated by GC, GC–MS and $^{13}$C NMR. α-Pinene, β-phellandrene, α-terpinyl acetate, Δ-3-carene, myrcene and α-phellandrene were found to be the main constituents. The results were submitted to cluster analysis and discriminant analysis which allowed two groups of essential oils to be distinguished with respect to the content of α-pinene, β-phellandrene and α-terpinyl acetate. © 2001 Elsevier Science Ltd. All rights reserved.

*Keywords:* *Juniperus phoenicea* subsp. *turbinata*; Cupressaceae; Essential oil; GC; GC–MS; $^{13}$C NMR; Statistical analysis; Intraspecific variability

## 1. Introduction

The genus *Juniperus* consists of approximately 60 species growing in the Northern Hemisphere and divided into three sections: *Caryocedrus, Juniperus* ( = *Oxycedrus*) and *Sabina*, the third being the most important one which comprises *Juniperus*

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*phoenicea* L. Recently, Adams (1999) showed on the basis of essential oil composition and RAPD DNA fingerprinting that *J. phoenicea* is distinguishable from other species of section *Sabina*.

*J. phoenicea* L. (Cupressaceae) is a shrub or a small tree from the Mediterranean region (Bonnier and Douin, 1990). Two subspecific taxa are clearly defined considering distinctive botanical features — not always well conspicuous — and phytogeographic distribution: *J. phoenicea* subsp. *phoenicea* L. characterized by globose female cones and obtuse or subacute scale-leaves, occurring in the inner mainlands and *Juniperus phoenicea* subsp. *turbinata* (Guss.) Parl. Nyman. ( = *J. phoenicea* subsp. *lycia* Auct. = *J. turbinata* Guss.) having elongated female cones and acute scale-leaves, occurring in coastal sites (Franco, 1964). According to phytogeographical repartition (Franco, 1964; Paradis, 1991) only the second subspecies grows wild in Corsica.

There is some controversy in the intraspecific treatment of *J. phoenicea*. Several local forms or biochemical pattern types were reported as subspecies or varieties, such as *J. phoenicea* subsp. *eu-mediterranea* Lebr. & Tiv. based on the prodelphinidine/procyanidine ratio in leaves (Lebreton and Thivend, 1981; Lebreton, 1983) but treated by Adams et al. (1996) as conspecific with *J. phoenicea* subsp. *turbinata.*

Essential oil of *J. phoenicea* is obtained by hydrodistillation of leaves, fruits or wood. There are few reports on the composition of *J. phoenicea* leaf oil, even though they concerned plants from various origins and a limited number of samples were analysed every time. The first studies date back to 1956 and 1973 and reported monoterpene-rich oils (subspecies not reported) (Gil de Meister and Hoffman, 1956; Banthorpe et al., 1973). More recently, Vidrich and Michelozzi (1993) reported 1,8-cineole, α-pinene and borneol as major components for an oil from Italy (subspecies not reported). Adams et al. (1996) studied samples from different origins and different subspecies. They reported the following compositions: α-pinene for *J. phoenicea* (*sensu stricto*) from inland Greece and Spain and α-pinene/β-phellandrene/α-terpinyl acetate for *J. phoenicea* var. *turbinata* from coastal Spain and *J. phoenicea* subsp. *eu-mediterranea* from coastal Portugal. In this study the authors emphasized the similarity of the essential oil composition of var. *turbinata* and subsp. *eu-mediterranea* and suggested that these taxa are conspecific.

*J. phoenicea* berry oils are characterized by a high content of α-pinene, whatever the origin (Fernandes-Costa and Cardoso do Vale, 1953–1954; Gil de Meister and Hoffman, 1956; Proença da Cuhna et al., 1977; De Pascual Teresa et al., 1981; Vidrich and Michelozzi, 1993; Falchi Delitala, 1980; Lawrence, 1989). The wood oil contains thujopsene and cedrol as major components (Adams, 1991), whereas pyrolitic oil is characterized by the predominance of α-cedrene (Chalchat et al., 1990). In addition, acidic and neutral diterpenes (Tabacik and Poisson, 1971; Tabacik and Laporthe, 1971; De Pascual Teresa et al., 1978a,b; Dawidar et al., 1991; San Feliciano et al., 1988, 1992, 1993) have been identified in solvent extracts of leaves and berries of *J. phoenicea.*

It appears from literature data that leaf oil of *J. phoenicea* has several compositions. Conversely, the α-pinene/β-phellandrene/α-terpinyl acetate composition was the only one reported for *J. phoenicea* subsp. *turbinata*. The purpose of this study was to determine if the same composition was obtained from *J. phoenicea* subsp. *turbinata*

from Corsica and to investigate if chemical variability occurred on the well-delimited territory of the island. We planned to collect individual plants from locations covering the geographic range of *J. phoenicea* subsp. *turbinata* in Corsica.

## 2. Materials and methods

### 2.1. Plant material

*J. phoenicea* subsp. *turbinata* grows wild in different locations in Corsica as reported by Paradis (1991). In order to study the chemical variability of leaf oils in a delimited area with constant pedoclimatic conditions, we collected 10 samples of single plants in the three most important stations (Fig. 1): Barcaggio (samples 1–10), Bonifacio (11–20), Ajaccio (21–30). Twenty samples were collected in the other locations (Fig. 1): Porto-Vecchio (31–38), Campomoro (39–43), west side of Cape Corsica (44–50). So, all the locations were investigated.

### 2.2. Sampling and leaf essential oils

Leaves were collected from many parts around and at different heights of the plants during the period of May–September, 1998 and submitted to hydrodistillation for 3 h

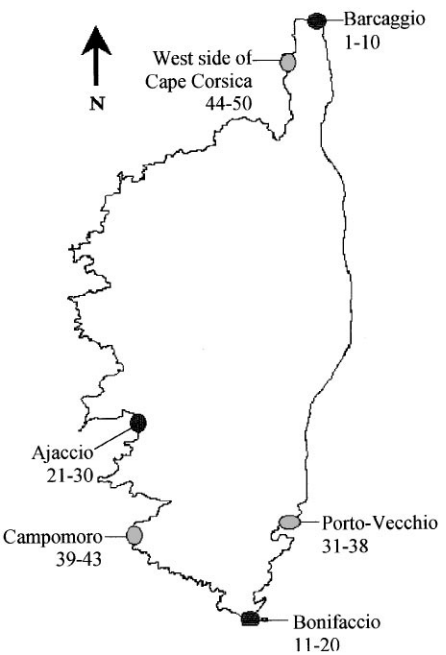


Fig. 1. Sampling of *Juniperus phoenicea* subsp. *turbinata* leaves from Corsica (dark grey: the three most important locations; light grey: other locations).

using a Clevenger-type apparatus. Essential oil yield, ranged between 0.1 and 0.7% (w/w, from fresh material).

### 2.3. GC, GC/MS and Carbon-13 NMR analyses

*Analytical GC*: GC analysis was performed using a Perkin-Elmer Autosystem apparatus equipped with two flame ionization detectors (FID), and fused capillary columns (50 m × 0.22 mm i.d., film thickness 0.25 μm), BP-1 (polydimethylsiloxane) and BP-20 (polyethyleneglycol). The oven temperature was programmed from 60 to 220°C at 2°C/min and then held isothermal (20 min); injector temperature: 250°C (injection mode: split 1/60); detector temperature: 250°C; carrier gas: helium.

*GC/MS*: GC–MS was performed with a Hewlett-Packard 6890 gas chromatograph, equipped with a polydimethylsiloxane fused-silica column (30 m × 0.25 mm i.d., film thickness 0.25 μm, HP19091Z-433.) interfaced with a Hewlett-Packard mass selective detector 5973 operated by HP Enhanced ChemStation software, version A.03.00. The oven temperature was programmed from 70 to 220°C at 3°C/min and then held isothermal (15 min); injector temperature: 250°C; carrier gas: helium, adjusted to a linear velocity of 30 cm/s; split 1/40; interface temperature: 250°C; MS source temperature: 230°C; MS quadrupole temperature: 150°C; ionization energy: 70 eV; ionization current: 60 μA; scan range: 35–350 u; scans/s: 4.51.

*$^{13}C$ NMR spectra*: NMR spectra were recorded on a Bruker AC 200 Fourier transform spectrometer, operating at 50.323 MHz, equipped with a 10 mm probe in deuterated chloroform (around 200 mg of oil in 2 mL of $CDCl_3$), with all shifts ($\delta$) referred to internal tetramethylsilane (TMS). Parameters: pulse width (PW): 5.0 μs (flip angle 45°); acquisition time: 1.3 s and relaxation delay $D_1$: 2 s (total recycling time 3.3 s) for 32 K data table with a spectral width (SW) of 12 500 Hz (250 ppm); composite phase decoupling (CPD) of the proton band; digital resolution: 0.763 Hz/pt; 5000 scans were accumulated for each sample. An exponential multiplication of the free induction decay with the line broadening of 1.0 Hz was applied before Fourier transformation.

### 2.4. Identification of components

$^{13}C$ NMR was carried out on the whole sample, without previous separation of the components, following the pioneering work done by Formàcek and Kubeczka (1982) and according to an experimental procedure and a computerized method developed in our laboratory (Tomi et al., 1995). The components were identified by comparison of the values of the carbon chemical shifts in the mixture spectrum with those of reference spectra compiled in a computerized data bank. Each compound is identified by taking into account three parameters, directly available from the computer program, (i) the number of observed signals with respect to that expected, (ii) the difference between the chemical shift of each signal in the mixture and in the reference ($\Delta\delta$), (iii) the number of overlapped signals of carbons belonging to two components which possess fortuitously the same chemical shift.

*GC*: The components were identified by comparison of their retention indices on polar and apolar columns with those of authentic samples. Retention indices were determined relative to retention times of a series of *n*-alkanes with linear interpolation ("Target Compounds" software from Perkin-Elmer). The relative proportions of the essential oil constituents were expressed as percentages obtained by peak-area normalization and no correcting factor has been applied.

*GC/MS*: Identification of the individual components was made by comparison of the acquired spectra with corresponding data from authentic samples or with literature data (Joulain and König, 1998; McLafferty and Stauffer, 1989) after a preliminary computer matching into a commercial mass spectra library.

### 2.5. Data analyses

The data were processed by Cluster Analysis using hierarchical clustering (Ward's technique and Euclidean distance measure) and were submitted to Discriminant Analysis, using the *xl*STAT-Pro software (Thierry Fahmy, France).

## 3. Results and discussion

Fifty samples of essential oil obtained from plant material were analysed by GC, GC/MS and carbon-13 NMR. Sixty-one components accounting for 95.1 to 95.9% of

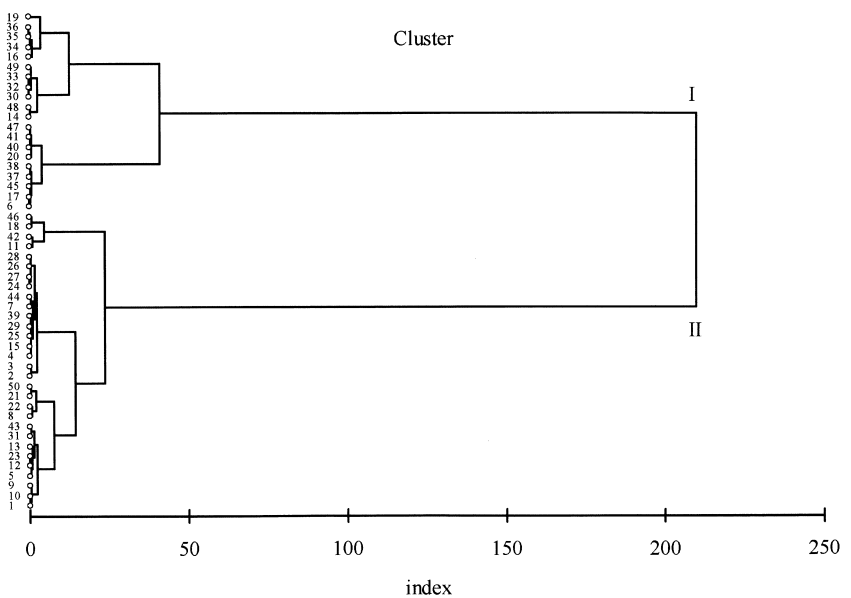


Fig. 2. Dendrogram obtained from the cluster analysis of 50 samples of *Juniperus phoenicea* subsp. *turbinata* from Corsica. Samples are clustered using Ward's technique with an Euclidean distance measure.

the total oil were identified (Table 1). Thirty components over 0.4% representing from 80.3 to 95.8% of the whole chemical composition were taken into account for statistical analysis. The dendrogram (Fig. 2) suggested the existence of two clusters (clusters I and II). Discriminant analysis (DA) (Figs. 3 and 4) confirmed this clustering and the two-dimensional axial system originated in the DA distinguished the different types of essential oils based on the contents of α-pinene, β-phellandrene and α-terpinyl acetate.

The essential oils of cluster I (40% of the samples) are characterized by a high content of α-pinene (mean 58.7%, S.D. = 9.2) (Fig. 5). The chemical composition of samples of cluster II (60% of the samples) is characterized by the weakest content of α-pinene (mean 33.0%, S.D. = 5.6) and a significant content of β-phellandrene (mean 21.1%, S.D. = 4.1) and α-terpinyl acetate (mean 8.2%, S.D. = 4.6) (Fig. 5). So, the leaf oils of *J. phoenicea* subsp. *turbinata* from Corsica exhibit a chemical variability with two clear composition patterns: α-pinene and α-pinene/β-phellandrene/α-terpinyl acetate. Both composition patterns are present in all the stations with different ratios although no relation between the composition of the oils and the habitat can be established. For instance, samples from Barcaggio and Ajaccio stations exhibit a ratio of cluster I/cluster II = 1/9 while samples from from Porto-Vecchio have a repartition of cluster I/cluster II = 7/1 although all these plants grow on sands (coastal fore-dunes). Conversely, samples from Bonifacio are equally distributed in the two clusters (cluster I/cluster II = 5/5), all plants occurring on cliffs. The compositions of two representative samples belonging to clusters I and II, respectively, are reported in Table 1.

The mean chemical composition determined for cluster II is close to those reported for oils of subspecies *turbinata* from Spain and Portugal (named *eu-mediterranea* in the

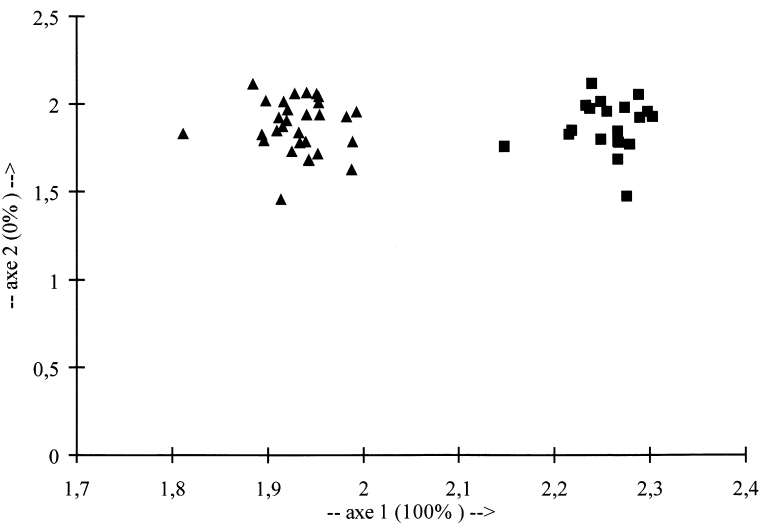


Fig. 3. Discriminant analysis scatterplot of 50 samples of *Juniperus phoenicea* subsp. *turbinata* from Corsica. (■) Cluster I, (▲) Cluster II.

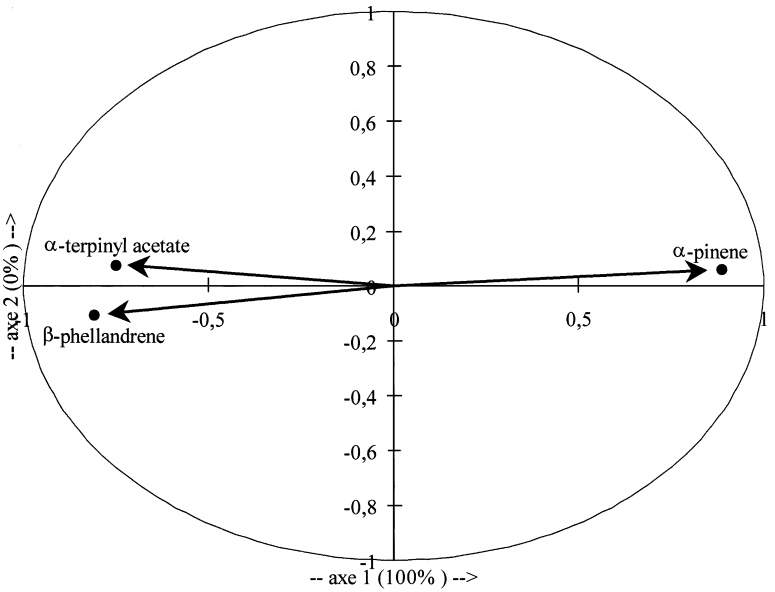


Fig. 4. Discriminant analysis scatterplot of the oil constituents.

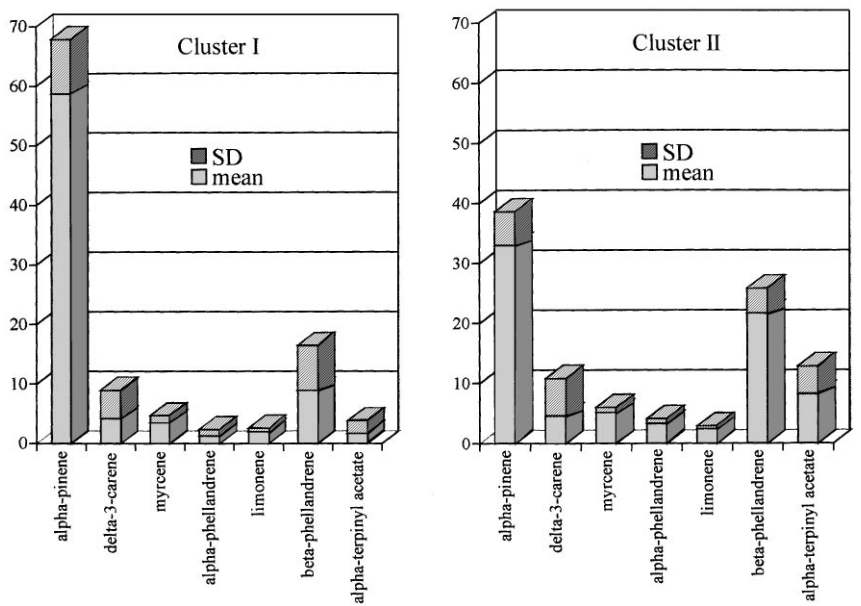


Fig. 5. Grey: mean percentage of major components, crosshatched: standard deviation.

Table 1
Chemical composition of two representative samples of *Juniperus phoenicea* subsp. turbinata leaf oil from Corsica[a]

| Constituents | BP-1 | Sample 35 (Cluster I) | Sample 15 (Cluster II) |
|---|---|---|---|
| Tricyclene | 920 | 0.2 | 0.1 |
| α-Pinene | 931 | **59.5** | **31.1** |
| Camphene | 943 | 0.3 | 0.2 |
| β-Pinene | 970 | **1.0** | **1.2** |
| Myrcene | 979 | **3.2** | **6.1** |
| 2-Carene | 996 | 0.1 | 0.4 |
| α-Phellandrene | 997 | **0.4** | **4.0** |
| Δ-3-Carene | 1005 | **6.5** | **3.1** |
| α-Terpinene | 1008 | 0.1 | 0.2 |
| p-Cymene | 1011 | **1.0** | **1.0** |
| Limonene | 1020 | **1.5**[b] | **2.2**[b] |
| β-Phellandrene | 1021 | **4.9**[b] | **23.8**[b] |
| γ-Terpinene | 1047 | 0.4 | 0.3 |
| Octanol | 1052 | tr | — |
| Fenchone | 1066 | 0.2 | 0.1 |
| Cymenene | 1071 | 0.1 | — |
| Terpinolene | 1078 | **0.6** | **1.8** |
| Linalool | 1081 | **0.4** | — |
| *cis*-Rose oxide | 1096 | tr | tr |
| α-Campholenal | 1102 | 0.4 | 0.1 |
| 2-Cyclohexen-1-ol | 1106 | — | 0.4 |
| Camphor | 1123 | **0.6** | — |
| *Trans*-Pinocarveol | 1125 | 0.1 | — |
| *cis*-Verbenol | 1127 | 0.4 | 0.4 |
| Iso-Borneol | 1142 | — | 0.1 |
| *p*-Mentha-1,5-dien-8-ol | 1143 | 0.3 | 0.1 |
| Borneol | 1148 | 0.4 | — |
| Cryptone | 1157 | 0.2 | 0.3 |
| *p*-Cymene-8-ol | 1158 | — | 0.2 |
| Terpinen-4-ol | 1161 | 0.1 | 0.3 |
| α-Terpineol | 1172 | **0.7** | **2.4** |
| Verbenone | 1184 | 0.1 | 0.1 |
| *trans*-Piperitol | 1187 | — | 0.1 |
| *trans*-Carveol | 1207 | 0.2 | 0.1 |
| Citronellol | 1208 | 0.1 | **1.1** |
| Geraniol | 1232 | — | 0.1 |
| Piperitone | 1233 | 0.1 | **2.6** |
| Linalyl acetate | 1240 | 0.1 | **0.5** |
| Iso-Pulegyl acetate | 1268 | — | 0.3 |
| α-Terpinyl acetate | 1332 | — | **9.8** |
| α-Cubebene | 1350 | 0.1 | — |
| α-Copaene | 1379 | 0.2 | — |
| β-Bourbonene | 1386 | 0.1 | — |
| β-Elemene | 1388 | 0.2 | — |
| E-Caryophyllene | 1424 | **1.1** | 0.2 |
| α-Humulene | 1453 | **0.7** | tr |
| α-Amorphene | 1470 | 0.8 | — |
| Germacrene D | 1478 | **0.9** | 0.2 |

Table 1 (*continued*).

| Constituents | BP-1 | Sample 35 (Cluster I) | Sample 15 (Cluster II) |
|---|---|---|---|
| Bicyclosesquiphellandrene[c] | 1489 | 1.1 | tr |
| α-Muurolene | 1494 | **0.4** | tr |
| γ-Cadinene | 1508 | **0.6** | tr |
| δ-Cadinene | 1516 | **1.0** | 0.2 |
| *cis*-Calamenene | 1519 | 0.3 | — |
| Elemol | 1533 | 0.1 | tr |
| Germacrene B | 1554 | 0.1 | 0.1 |
| Caryophyllene oxide | 1576 | **0.6** | 0.1 |
| Humulene epoxide | 1582 | 0.4 | — |
| Epi-Cubenol | 1618 | **1.5** | 0.1 |
| τ-Muurolol | 1638 | 0.1 | — |
| α-Cadinol | 1640 | 0.3 | 0.2 |
| Manoyl oxide | 1996 | 0.4 | — |

[a]Components were identified by GC-retention indices on two columns, GC/MS and $^{13}C$ NMR (bold numbers). Compositional values less than 0.05% are denoted as trace (tr). Order of elution and percentages of individual components are given in BP-1 column.

[b]Order of elution and percentages of individual components are given in BP 20 column.

[c]Correct epimer not identified.

last case) (Adams et al., 1996). The mean chemical composition of the samples belonging to cluster I (α-pinene-rich oils not yet reported for *J. phoenicea* subsp. *turbinata*) is close to those reported for *J. phoenicea* (*sensu stricto*) oils from Greece and Spain (Adams et al., 1996) and for *J. phoenicea* (subspecies not specified) oils (Gil de Meister and Hoffman, 1956; Banthorpe et al., 1973).

These results can be explained as follows. If only the subspecies *turbinata* occurs in Corsica, then this taxon exhibits chemical variability. However, from our results, we cannot exclude the presence of two subspecies growing in the same habitat and insufficiently differentiated by botanical features.

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